Regulation of the Oxidative Stress Response by ZTF-17 in *Caenorhabditis elegans*

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ABSTRACT

Reactive oxygen species (ROS) are common by-products of normal cellular metabolism and have important physiological roles in cell signaling and homeostasis. However, when there is an excess production of ROS, a dangerous condition known as oxidative stress (OS) occurs where by the body is overwhelmed and unable to detoxify these free radicals. ROS readily react with other macromolecules causing damage to DNA, lipids and proteins, severely compromising cell health and contributes to the onset of age-associated diseases. Many organisms have devised antioxidant systems to protect themselves and in *Caenorhabditis elegans*, two important transcription factors, SKN-1/Nrf2 and DAF-16/FOXO promote the expression of stress resistance genes. Phase II detoxifying genes such as *gst-4* is expressed through SKN-1, while *sod-3* is under DAF-16 control, and both confer stress resistance when activated. When an RNAi against transcription factor ZTF-17 was used, enhanced *gst-4p::gfp* expression was observed suggesting that ZTF-17 possessed repressor like functions. ZTF-17 is uncharacterized but its mammalian homolog, ZFP42/REX1, is a pluripotency factor that represses transcription of the *Xist* gene during X-chromosome inactivation. We observed that *ztf-17(tm963)* deletion mutants had increased *gst-4p::gfp* and *sod-3p::gfp* expression and confirmed by qRT-PCR that mRNA levels of both genes were significantly enhanced when compared to wild type. Although the detoxification process exists, the mechanism by which the levels of free radicals are regulated and the molecular players involved in maintaining proper function under OS remains unclear. Our lab aims to investigate ZTF-17's function along with characterizing its role as a potential negative regulator of SKN-1 and DAF-16 target genes implicated in the OS response, lifespan and longevity.

PATHWAYS

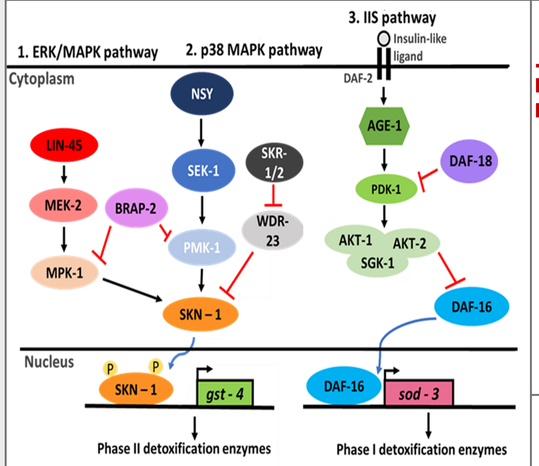

Figure 1. Schematic representation of the ERK/MAPK, p38 MAPK and DAF-2 Insulin-like pathways that regulates SKN-1 and DAF-16 during oxidative stress in *C. elegans*[1-4].

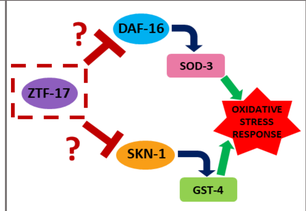

Figure 2. The proposed model for ZTF-17 that negatively regulates the activation of oxidative stress response genes. The effect of ZTF-17 on SKN-1 and DAF-16 requires further research.

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RESULTS

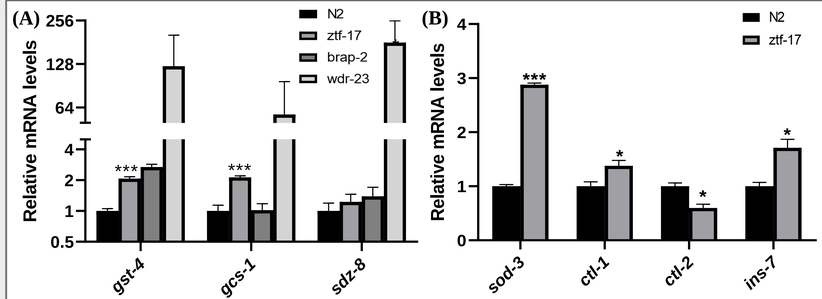


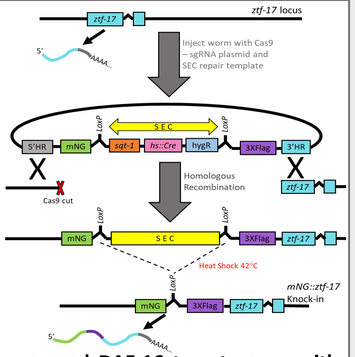
Figure 3. qRT-PCR monitoring amplification of antioxidant genes. (A) Relative mRNA levels of phase II detoxification genes show that *gst-4* and *gcs-1* expression levels are higher in *ztf-17(tm963)* worms. (B) Relative mRNA levels of DAF-16 target genes show that *sod-3, ctl-1* and *ins-7* expression levels are higher in *ztf-17(tm963)* mutants. Worms were synchronized to L4 then harvested for RNA isolation. qRT-PCR was preformed using Rotor Gene Q (Qiagen Inc.) *act-1* reference gene was used as the internal control (n=3 trials where *** P < 0.001, ** P < 0.01, * P < 0.05). Error bars represent SEM and p-values were derived using the Holm-Sidak method for statistics.

FUTURE WORK

❖**Determine ZTF-17 localization** – Using SapTrap assembly to produce a CRISPR/Cas-9 mediated mNG::*ztf-17* fluorescent protein knock-in as a gene tagging strategy for generating transgenic worms[5].

❖**Carry out ChIP-seq analysis for ZTF-17** – Possibility that ZTF-17 directly interacts with promoters to repress transcription of detoxification genes.

❖**Link promoters to luciferase to study gene transcription levels** – Observe whether ZTF-17 attenuates SKN-1 and DAF-16 transcriptional activity.



❖**Validate that *ztf-17* enhances PII detoxification genes and DAF-16 target genes with RNAi** – Using qPCR, gene amplification will elucidate if ZTF-17 has a specific or broad effect on antioxidant gene expression.

❖**DULIP Assay to map potential protein interactions between ZTF-17 and DAF-16A** – construct PA-RL-DAF16A bait and FL-ZTF17 prey fusion proteins; luminescence-based quantification to determine interactions.

❖**Microarray analysis of N2, *daf-2* and *ztf-17* mutants** – Upregulation or downregulation of genes in these strains may help to identify potential candidates involved in the increased oxidative stress response when *ztf-17* is knocked down.

RESULTS

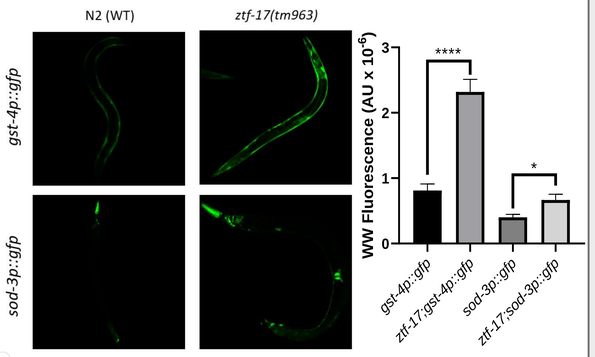


Figure 4. Confocal images of Whole Worm (WW) fluorescence on *ztf-17(tm963)* showed an increase of ~ 2.86 and ~1.66 times that of wildtype worms for *gst-4p*::GFP and *sod-3p*::GFP respectively. Image quantification by ImageJ software, Welch's t-test was used for statistics where n=16 for each sample and **** P < 0.0001, *** P < 0.001 ** P < 0.01 and * P <0.05.

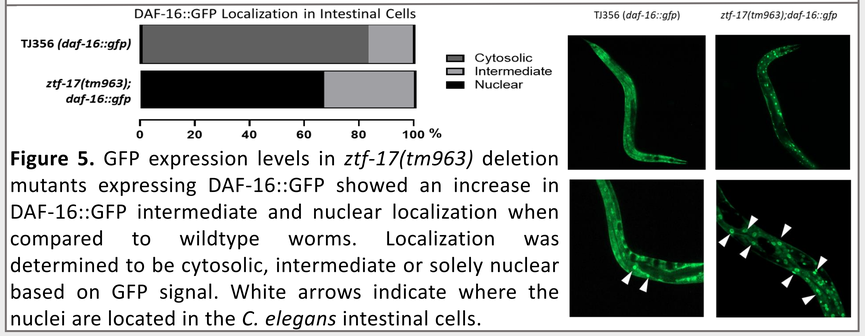


Figure 5. GFP expression levels in *ztf-17(tm963)* deletion mutants expressing DAF-16::GFP showed an increase in DAF-16::GFP intermediate and nuclear localization when compared to wildtype worms. Localization was determined to be cytosolic, intermediate or solely nuclear based on GFP signal. White arrows indicate where the nuclei are located in the *C. elegans* intestinal cells.

SUMMARY

❖We observed that *ztf-17(tm963)* mutants had increased *gst-4p::gfp* and *sod-3p::gfp* expression and confirmed by qRT-PCR that mRNA levels of both genes were significantly enhanced when compared to wild type.

❖qRT-PCR monitoring other PII and DAF-16 target genes suggests ZTF-17 has a role in modulating their expression.

❖Increased DAF-16::GFP nuclear localization is indicative of increased DAF-16 activity in *ztf-17(tm963)* mutants.

REFERENCES AND ACKNOWLEDGEMENTS

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